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MPScope: A versatile software suite for multiphoton microscopy

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Abstract

MPScope is a software suite to control and analyze data from custom-built multiphoton laser scanning fluorescence microscopes. The acquisition program MPScan acquires, displays and stores movies, linescans, image stacks or arbitrary regions from up to four imaging channels and up to two analog inputs, while plotting the intensity of regions of interest in real-time. Bidirectional linescans allow 256 × 256 pixel frames to be acquired at up to 10 fps with typical galvanometric scanners. A fast stack mode combines movie acquisition with continuous *z*-focus motion and adjustment of laser intensity for constant image brightness. Fast stacks can be automated by custom programs running in an integrated scripting environment, allowing a 1 mm^3 cortical volume to be sampled in 1 billion voxels in approximately 1 h. The analysis program MPView allows viewing of stored frames, projections, automatic detection of cells and plotting of their average intensity across frames, direct frame transfer to Matlab, AVI movie creation and file export to ImageJ. The combination of optimized code, multithreading and COM (Common Object Model) technologies enables MPScope to fully take advantage of custom-built two-photon microscopes and to simplify their realization.

Keywords: Femtosecond laser; Two-photon; Electrophysiology; Software; Scripting; Calcium imaging; Histology

1. Introduction

Multiphoton microscopy (Denk et al., 1990) was first applied to neurobiology more than a decade ago (Denk et al., 1994) and has since acquired a prominent status among neuroscience methods (for a review, see Nguyen et al., in press). The advantages of multiphoton laser scanning microscopes (MPLSMs) include deep penetration (100–1000 μm) into highly scattering neural tissues (Helmchen and Denk, 2005), micron to sub-micron resolution and optical *z*-sectioning, as well as reduction of fluorophore bleaching and/or phototoxic damages.

Custom-built MPLSMs (e.g. Fan et al., 1999; Mainen et al., 1999; Majewska et al., 2000; Nguyen et al., 2001; Nikolenko et al., 2003; Roorda et al., 2004; Tan et al., 1999; Tsai et al., 2002) are not only considerably cheaper than commercial microscopes (by almost a factor of two) but can also be specifically designed for a particular purpose such as in vivo recordings (Tsai et al., 2002). One limitation of this approach is that programs controlling custom MPLSMs (e.g. Nguyen et al., 2001; Pologruto et al., 2003; Tsai et al., 2002) incorporate only a certain number of features from an ideal "wish list" of requirements for neuroscience.

The most important feature is a high frame rate that will enable the capture of fast functional events such as variations in intracellular calcium concentrations as well as the possibility to provide a near real-time assessment of cellular responses as the experiment unfolds. This can be achieved by first drawing areas of interest encompassing select neurons and then by having the program plot during the acquisition session the average pixel intensity of these areas for each frame as a time series in an oscilloscope-like display. Also, simultaneous frame acquisition, display and disk streaming is of considerable importance and thus precludes the use of a "blind" mode where frames are captured and stored without any form of visual feedback.

Further, the need to coordinate electrophysiological recordings and image acquisition is important for many functional experiments. One solution is to centralize the control of most electrophysiological operations in the scanning program to ensure proper synchronization of the analog and video streams.

Another requirement of MPLSM systems is the ability to generate, magnify and rotate laser scan patterns in order to bring the scanning field into a favorable orientation to image

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particular structures or processes such as dendrites or blood vessels. This feature is critical when measuring cerebral blood flow (Kleinfeld et al., 1998), since the method depends on aligning linescans on top of linear portions of blood vessels. Some multiphoton microscopes (e.g. Tsai et al., 2002) depend on a custom-designed electronic circuit based on an embedded DSP or PIC processor to rotate and magnify the scan pattern. These devices increase the overall complexity of the microscope and are difficult to replicate, maintain and upgrade. The phenomenal computing power now available in virtually all current personal computers should, in principle, be largely sufficient to allow the MPLSM program to undertake the functions of these devices.

Perhaps the most difficult requirement for MPLSM software is the possibility to use the same program in widely different experiments ranging from time-lapse imaging (Ruthazer and Cline, 2002) to histology (Tsai et al., 2003). MPLSM programs can, in theory, be customized by modifying their source code. However, this solution can challenging even when the programs are written in the Matlab (MathWorks, Natick, MA) or Labview (National Instruments, Austin, TX) programming languages. As an alternative, customization and automation of repetitive tasks can be achieved by providing users with the possibility to write small programs called scripts, usually in an easy to learn programming language like Microsoft VBA (Visual Basic for Application) or VBScript (Visual Basic Scripting Edition). This approach is adopted in the commercial programs LaserSharp and LSM that control, respectively, the Radiance2100 MP from Bio-Rad (now Carl Zeiss CellScience) and the LSM 510 NLO (Zeiss) multiphoton systems, and also in the custom-build system described by Nikolenko et al. (2003). Scripting based on the Microsoft ActiveX standard has the advantage of allowing users to include in their scripts some of the many ActiveX software components already written or to tap into functionalities present in existing programs, thus creating script-based "meta-applications" (Nguyen and Miledi, 2003).

In this paper we present MPScope, a suite of turnkey applications that fulfills the requirements outlined above. MPScan, the frame acquisition program, is flexible enough to control any MPLSM based on generic scanning and data acquisition hardware. MPView is designed to analyze the data generated by MPScan. We describe the design principles we adopted for MPScope and present benchmark data based on a novel imaging scheme allowing deep z-stacks to be taken almost up to an order of magnitude faster than with a previous program written in LabView.

2. Material and methods

2.1. Software development

MPScope was developed in Object Pascal using the Delphi 5 programming environment (Borland Corp., Scotts Valley, CA) on a Dell Inspiron 8000 laptop (Dell Corp., Austin, TX). MPScan ran on a dual 1 GHz processor Dell workstation with 1 GB RAM running Microsoft Windows 2000 Professional Edition (Microsoft Corp., Redmond, WA).

2.2. Software and hardware requirements

MPScope is designed for IBM-compatible personal computers running the Microsoft Windows 2000 or XP operating systems. As stand-alone compiled executables, MPScan and MPView do not depend on a run-time environment. MPScan requires National Instruments PCI-6110E card and the Traditional NI-DAQ library (v. 6.9 or higher), which is automatically installed when setting up the data acquisition board.

2.3. Multiphoton microscope

MPScan was tested on a femtosecond laser scanning fluorescence microscope instrument consisting of a Mira-Verdi pump-femtosecond laser combination (Coherent, Santa Clara, CA), fluorescence optics and detection electronics (Tsai et al., 2002). Fluorescence photons are detected by up to four imaging channels equipped with photomultipliers (PMTs) (Model R6357, Hamamatsu, Bridgewater, NJ). The PMT output currents are converted into voltages by locally designed feedback resistor-based preamplifiers. The resulting voltages are further amplified and either low-pass filtered or integrated by a custom four-channel pixel integrator. The integration time of this device is set by the analog conversion clock of the data acquisition board.

2.4. MPScan: hardware interfacing

MPScan was designed to be compatible with custom-made multiphoton microscopes such as the Denk/Svoboda design described in Pologruto et al. (2003) and the instrument described in Tsai et al. (2002). The program only requires that the scanning system consist of a pair of orthogonally mounted, servo-controlled galvanometric mirrors. The deflection of the laser beam, set by the angle of each mirror, is directly controlled by the analog outputs of the main data acquisition board (Fig. 1(A)). Our microscope and the one described in Pologruto et al. (2003) use two Model 6210 galvanometric mirrors controlled by MicroMax 670 servos (Cambridge Technologies, Cambridge, MA). The maximal voltage output from the board can be set by the user in MPScan to use galvanometric mirrors from other vendors that have different input voltage requirements (e.g. those from GSI Lumonics).

The PCI-6110E data acquisition board is also used to digitize the signals from up to four imaging channels. In case analog signals (e.g. electrophysiological data) need to be recorded simultaneously, the last two imaging channels can be used instead to sample analog data (Fig. 1(A)). An additional, optional PCI analog output board (National Instruments PCI-6711) can be controlled by MPScan. The board provides two analog output channels which can be used to control additional devices such as a Pockels cell to modulate the laser beam intensity (e.g. Nikolenko et al., 2003) or to generate stimulation waveforms for cellular amplifiers. The laser shutter can be turned on or off either by a digital output line from one of the PCI boards. Additional data acquisition boards can be